

## INFLUENCE OF AGE ON THE SUSCEPTIBILITY OF PIGLETS TO *ESCHERICHIA COLI* O138:F18

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### ABSTRAK

Penelitian secara *ex vivo* dan *in vivo* dilaksanakan untuk mengetahui pengaruh umur terhadap kerentanan babi atas diare akibat *E. coli* F18. Percobaan secara *ex vivo* menggunakan babi umur 2 dan 4 minggu, dilaksanakan berdasarkan 'porcine intestinal organ culture model'. Percobaan secara *in vivo* menggunakan babi umur 2 dan 3 minggu yang diberi susu pengganti dan diinfeksi dengan *E. coli* F18. Inokulasi dengan *E. coli* F18 pada kultur usus mengakibatkan peningkatan jumlah *E. coli* pada jaringan usus, namun jumlah *E. coli* tidak berbeda antara babi umur 2 dan 4 minggu. Bahan kering feses menurun, sedangkan proporsi babi yang menderita diare dan jumlah hemolitik *E. coli* pada feses meningkat setelah inokulasi, namun perbedaan akibat umur tidak terlihat pada percobaan secara *in vivo*. Interaksi antara umur dan hari pengambilan sampel terlihat pada jumlah total sel darah putih. Proporsi neutrofil menurun, sedangkan limfosit, sel darah merah, hemoglobin dan hematokrit meningkat setelah inokulasi, tetapi perbedaan antara kelompok umur tidak terlihat pada parameter tersebut. Sebagai kesimpulan, umur (2 dibandingkan dengan 3 atau 4 minggu) tidak mempengaruhi kerentanan babi terhadap diare akibat *E. coli* F18.

**Kata kunci:** babi, diare, *E. coli* F18, *ex vivo*, *in vivo*, kerentanan

### ABSTRACT

The *ex vivo* and *in vivo* studies were undertaken to examine age-effect on the susceptibility of pigs to *E. coli* F18-diarrhea. The *ex vivo* experiment was conducted with 2 and 4 weeks old pigs based on the porcine intestinal organ culture (PIOC) model. The *in vivo* experiment was performed with 2 and 3 weeks old pigs fed milk replacer and inoculated with *E. coli* F18. *E. coli* F18 inoculation to the intestinal cultures resulted in higher ( $P<0.01$ ) counts of *E. coli* associated to the intestinal tissue, but such difference was not observed between the 2 and 4 weeks old pigs. Faecal dry matter (DM) decreased ( $P=0.01$ ), whereas the proportion of pigs with diarrhea and faecal haemolytic *E. coli* counts increased ( $P<0.05$ ) following inoculation, however, the difference between the ages was not observed in the *in vivo* study. The interaction ( $P=0.01$ ) between age and sampling day was observed for the number of total white blood cells (WBC). The proportion of neutrophils decreased ( $P=0.01$ ), whereas the lymphocytes, red blood cells (RBC), hemoglobin (Hb) and hematocrit (Hct) increased ( $P<0.01$ ) after inoculation, but the differences between age groups were not observed regarding these variables. In conclusion, age (2 versus 3 or 4 weeks) did not influence the susceptibility of piglet to *E. coli* F18-diarrhea.

**Keywords:** *E. coli* F18, diarrhea, *ex vivo*, *in vivo*, piglet, susceptibility

## INTRODUCTION

Diarrhea in young pigs has been ascribed by the presence of enterotoxigenic *Escherichia coli* (ETEC) expressing F4 or F18 fimbriae. The association of ETEC to pig intestinal tissue (mucosa) is considered to be a prerequisite for the development of this intestinal disease, as it inflicts colonization of the intestine by ETEC, which in turn enable them to deliver enterotoxin leading to diarrhea (Fairbrother *et al.*, 2005). In contrast to ETEC F4, ETEC with F18 fimbriae do not cause diarrhea in neonatal and piglets below 3 weeks of age, even when piglets are genetically susceptible to ETEC F18 (Fairbrother *et al.*, 2005; Coddens *et al.*, 2007). The susceptibility of pigs to ETEC F18-diarrhea is determined by the presence and activity of F18-receptor on the small intestinal epithelium that is controlled by  $\alpha(1,2)$ -fucosyltransferase gene (FUT1), and that the FUT1 gene is not expressed in the intestine of pigs before 3 weeks of age (Coddens *et al.*, 2007). In contrast to this, a more recent study showed that FUT1 expression was detected, with no apparent differences in terms of the levels, in the proximal and distal intestine of neonatal and piglets weaned at 28 days of age (Jensen *et al.*, 2012).

Suckling piglets or piglets weaned to milk replacer (piglets younger than 3 weeks of age; note that pigs are normally weaned to solid feed at 4 weeks of age or more) are considered as a good animal model for studying the pathophysiology of diarrhea caused by bacteria in the infants (Correa-Matos *et al.*, 2003). The discrepancy between the studies of Coddens *et al.* (2007) and Jensen *et al.* (2012) regarding the expression of FUT1 gene in the pig intestine as previously mentioned has, however, raised some doubt with regard to the susceptibility of the piglets to *E. coli* F18-diarrhea before 3 weeks of age. To deal with this, an *ex vivo* experiment with 2 and 4 weeks old pigs, and an *in vivo* experiment involving 2 and 3 weeks old pigs were conducted in the present study. The objective of this study was to examine the *E. coli* F18 association *ex vivo* to the intestinal tissue of pigs at 2 and 4 weeks of age, and the occurrence of *E. coli* F18-diarrhea *in vivo* in 2 and 3 weeks old pigs weaned to milk replacer. The hypothesis of the study was that intestinal tissue of 4 weeks old pigs would be more colonized by *E. coli* F18 than that of 2 weeks, and pigs challenged with *E. coli* F18 would be more susceptible to diarrhea when weaned at 3 weeks of age compared to

weaning at 2 weeks of age.

## MATERIALS AND METHODS

### *Ex vivo* study

#### *Porcine intestinal organ culture model*

Piglets (2 weeks old, N=4; 4 weeks old, N=4) used in the *ex vivo* study were genetically susceptible to ETEC F18 (originating from 2 sows tested homozygote carriers of the dominant gene encoding for the intestinal F18 fimbriae receptors). The study was based on the PIOC model described by Sugiharto *et al.* (2012) with few modifications. In brief, piglets were taken from their dams and sacrificed, and immediately intestinal segments (2 segments representing of jejunum; ~15 cm for each) were collected aseptically and immersed in Dulbecco's modified Eagle's medium (DMEM) on ice. Polyethylene tubing (Siltube; Eurpharm; 6 mm in diameter) was inserted into either end of the segment and tied with a suture to keep the tubing in place. The tissue was washed with 50 mL PBS (pH 7.2) using a FillMaster pump (Type 311; Delta Scientific Medical; flow rate of 7.7 cm/s). The other end of the segment was tied, 10 mL of DMEM alone (control) or DMEM containing *E. coli* F18 was inoculated, and the segment was sealed with Teflon plug (5 mm in diameter). The segment was immersed in DMEM in a 300-mL infusion bottle in a shaking water bath at 37°C, removed after 1.5 h and washed with 50 mL of PBS after removing the content. The segment was weighed and homogenized (Janke-Kunkel Ultra-Turrax T25 homogenizer, the Netherlands) for 20 s in PBS. *E. coli* was enumerated on McConkey agar (Merck KGaA, Darmstadt, Germany) after aerobic incubation at 37°C overnight.

#### *E. coli* F18 inoculum preparation

*E. coli* O138:F18 inoculum was prepared by retrieving the bacterial culture stored at -80°C, streaking on blood agar (BA; Oxoid, Deutschland GmbH, Wesel, Germany) and culturing at 37°C for 18 h. A loopful of *E. coli* F18 colony was taken from BA and suspended in 4 mL PBS. The suspension (0.1 mL) was poured onto iso-sensitest agar (Oxoid) and incubated at 37°C overnight. PBS (10 mL) was poured on the incubated plate, and the agar surface was gently rubbed with a sterile Drigalski spatula to remove the bacterial colonies from the agar plate. The bacterial suspension was transferred to the sterile tube and diluted 1:20 (vol/vol) with DMEM to get the

inoculum of  $5 \times 10^8$  cfu/mL before use for inoculating the intestinal segments.

### ***In vivo study***

#### ***Experimental design***

The animal experiment was conducted according to the license obtained by the Danish Animal Experiments Inspectorate, Ministry of Food, Agriculture and Fisheries, Danish Veterinary and Food Administration. Twenty four piglets (originating from 3 sows) genetically susceptible to ETEC F18 were used. Within each litter, 3 pigs of similar weight were penned together in one of eight  $1.45 \times 1.70$  m<sup>2</sup> pens equipped with an automated wet feeder (Mambo Automix 25, Wit-Mambo Inc., Wommels, the Netherlands). From the weaning day (day 1 of experiment) and onward, 2 weeks (BW= $4.68 \pm 0.57$  kg and 3 weeks old ( $5.85 \pm 0.92$  kg) pigs were separated from their sows and fed milk replacer (Arla Foods Amba, Viby, Denmark). They were also provided 1 kg commercial produced creep feed (without zinc oxide) per-pen per-day which given in the morning and afternoon. The milk replacer was mixed with warm water (45°C) immediately before serving through the automated wet feeder, and the piglets were fed 4 times per-hour on the day (8 am to 7 pm) and once every hour on the night (25 g milk powder for each portion).

To perform an experimental-induction of *E. coli* F18-diarrhea, the piglets were orally inoculated with *E. coli* F18 through the milk replacer. *E. coli* O138:F18 inoculum was prepared (as described for the *ex vivo* study) for each inoculation. The bacterial suspension was transferred to the sterile tube and diluted 1:100 in PBS. Diluted bacterial suspension was suspended 1:100 in liquid milk replacer. Inoculation was conducted at 11 am by pouring of 150 mL milk replacer containing *E. coli* F18 inoculum on the through of the automated wet feeder during 3 subsequent days (day 2, 3 and 4 of the experiment). For each inoculation, the individual pig was scheduled to receive  $3 \times 10^8$  cfu *E. coli* F18 in 50 mL milk replacer. Milk replacer and creep feed were removed prior to inoculation, i.e., from 8 to 11 am, to assure that the piglets were willing to drink the milk replacer containing the challenge-inoculum. Immediately after the *E. coli* F18-milk suspension had been completely consumed, the automated wet feeder was turned on and creep feed was provided. The current study

did not include any piglets that were not inoculated with *E. coli* F18 as the control group. This was underlined by the previous study (Sugiharto *et al.*, 2014) whereby piglets genetically susceptible to *E. coli* F18 suffered from diarrhea after *E. coli* F18 inoculation, but not before the inoculation, indicating that these piglets are sensitive to *E. coli* F18.

#### ***Sample collection and analysis***

Blood was collected in EDTA-vacutainer by puncture from the jugular vein on day 1, 8 and 11. Rectal temperature was recorded at day 1, 3, 4, 5, 6, 8 and 10 of experiment. At the same time, faecal samples for bacteriological and DM analysis was obtained from the rectum of each piglet. Faecal score (1=hard, dry and cloddy, 2=firm, 3=soft with shape, 4=soft and liquid, 5=watery and dark, 6=watery and yellow, 7=foamy and yellow) was made, and diarrhea was defined for the score >3. The piglets were also evaluated daily for their health (0=healthy, and 1=unhealthy) and behaviour (0=active, and 1=listless).

For faecal bacteriology, 1 g faeces was suspended in peptone solution (1:10, wt/vol) and homogenized by bag mixer (BagMixer100, Interscience, St. Nom, France). Serial 10-fold dilution was prepared, 100 µL plated on BA and McConkey agar (Merck) for enumeration of haemolytic *E. coli* and total *coliform*, respectively, and incubated aerobically at 37°C overnight. Faecal DM was determined by freeze-drying the faeces samples to a constant weight. Immediately after collection, complete blood count test was conducted using hemocytometer (Cell-Dyne® 3500R, Abbott Diagnostics, Santa Clara, CA).

At day 11, all piglets were sacrificed using a captive bolt pistol, the small intestine was rapidly removed, and the length was measured and divided into 3 segments of equal length. From the middle of each segment, a 10 cm segment was obtained for measurements of intestinal dimensions (Zhang *et al.*, 1998). For enumeration of haemolytic *E. coli* and *coliform* bacteria associated with the intestinal mucosa, 5 cm segments were aseptically obtained from 50% (mid) and 90% (distal) of the small intestinal length. Each segment was washed-through with 50 mL sterile PBS, weighed, and homogenized by tissue homogenizer (Ultra-turrax T25, Janke & Kunkel IKA Labortechnik, Staufen, Germany) for 20 s in PBS. Final dilution of  $10^{-6}$  was prepared

from the homogenate, plated on BA and McConkey agar, and incubated aerobically at 37°C overnight.

### Statistical Analysis

Data obtained from the *ex vivo* study were analyzed using MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). Data analysis of the *in vivo* study included a total of 15 piglets (2 weeks old, N=6; 3 weeks old, N=9), as 9 piglets refused to drink the milk replacer throughout the experiment, and were thereby excluded from the analyses. Data on the proportion of pigs with diarrhea, health condition and behaviour of the piglets were analyzed using GLIMMIX procedure of SAS with days as repeated measurement. The MIXED procedure was used to analyze the data on faecal DM and microbial counts, rectal temperature, haematological parameters, intestinal dimensions and counts of bacteria associated with the intestine, with sampling days or intestinal segments as repeated measurement. In both studies, litters were treated as random effects and the individual segment (*ex vivo* study) or pig (*in vivo* study) was considered as the experimental unit. Interactions among the systemic effects were initially included in the statistical model, but the parameter was subsequently excluded when the interaction was not statistically significant. Bacterial counts, total WBC and RBC were log-transformed before analysis. The results are presented as LSMEANS and SE.

## RESULTS

### Ex vivo Study

The counts of *E. coli* associated with the

small intestinal tissue and content of piglets are presented in Table 1. Inoculation with *E. coli* F18 to the intestinal organ cultures resulted in higher ( $P<0.01$ ) numbers of *E. coli* associated to the intestinal tissue and content. However, there was no difference in *E. coli* counts between the 2 weeks and 4 weeks old pigs. No interaction ( $P>0.05$ ) between age of piglets and *E. coli* F18 inoculation was observed in the present *ex vivo* study.

### In vivo Study

The inoculation with *E. coli* F18 increased ( $P<0.05$ ) the proportion of piglets suffering from diarrhea and the content of haemolytic *E. coli* in the faeces. An interaction ( $P=0.02$ ) between weaning age and days of sampling was observed for the faecal DM. Whereas the faecal DM remained low after inoculation with *E. coli* in 3 weeks old pigs, the faecal DM of 2 weeks old pigs increased to the same values measured before inoculation at the end of experiment (Figure 1). The proportion of the piglets suffering from diarrhea, responses of health- and behaviour condition, the rectal temperature and the faecal bacteria counts did not differ between the age groups.

The data of haematological parameters are presented in Table 2. The interaction ( $P=0.01$ ) between the weaning age and sampling day was observed for the number of total WBC. The proportion of neutrophils to the total WBC decreased ( $P=0.01$ ), whereas the proportion of lymphocytes increased ( $P=0.01$ ) after inoculation. The inoculation increased ( $P<0.01$ ) the concentrations of RBC, Hb and Hct of the piglets, but the differences between age groups were not

Table 1. The Number of *E. coli* Associated with the Small Intestinal Tissue and Content of Piglets

	Age				SE	P-value*	
	2 weeks old		4 weeks old			Age	<i>E. coli</i>
<i>E. coli</i> F18 inoculation	–	+	–	+			
Intestinal tissue (log cfu/g)	4.99	7.56	4.62	7.67	0.28	0.59	<0.01
Intestinal content (log cfu/mL)	4.74	8.78	4.00	8.88	0.47	0.51	<0.01

\*There was no interaction ( $P>0.05$ ) between age of piglets and *E. coli* F18 inoculation. (–): intestinal organ cultures were not inoculated with *E. coli* F18, and used as control. (+): intestinal organ cultures were inoculated with *E. coli* F18. SE: standard error.

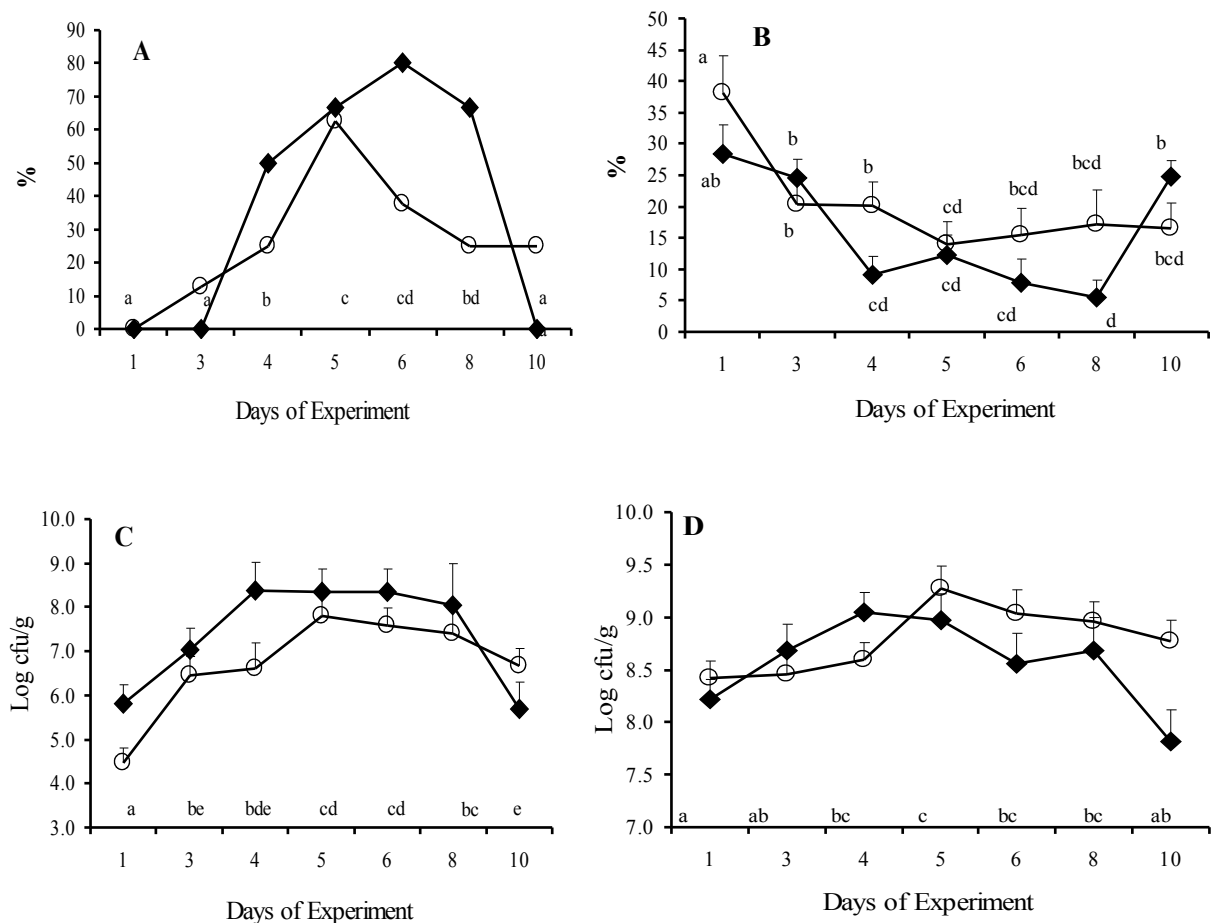


Figure 1. Proportion of pigs with diarrhea (A), DM of the faeces (B) and faecal shedding of haemolytic *E. coli* (C) and *coliform* (D) throughout the experiment. The inoculations with *E. coli* F18 were conducted on day 2, 3 and 4 of the experiment. The different letters along the X-axis in A, C and D indicate differences ( $P < 0.05$ ) among the days of experiment. There was no difference ( $P > 0.05$ ) between the age groups in A, C and D. The different letters in B indicate interaction ( $P < 0.05$ ) between the age of piglets and days of experiment.

observed regarding these variables. The counts of haemolytic *E. coli* and total *coliform* associated with the intestinal tissue did not differ between the age groups. The distal small intestine had higher total *coliform* ( $P < 0.01$ ) and numerically higher haemolytic *E. coli* than mid small intestine (Figure 2). Relative to the body weight of the piglets, the wet-weight of small intestine ( $12.86 \pm 0.77$  and  $13.87 \pm 0.46$  g/kg; LSMEANS and SE for 2 and 3 weeks old pigs, respectively), dry-weight of the intestinal mucosa ( $0.38 \pm 0.05$  and  $0.34 \pm 0.03$  g/kg) and the percentage of mucosa to intestinal tissue ( $23.94 \pm 3.44$  and  $20.82 \pm 2.07\%$ ) were not different between the ages.

## DISCUSSION

The *ex vivo* and *in vivo* studies were carried out to elucidate the effect of age on the susceptibility of pigs to ETEC expressing F18 fimbriae. Owing to the disputable studies between Coddens *et al.* (2007) and Jensen *et al.* (2012) as mentioned earlier, the current studies are thereof important to infer the possibility of using suckling piglet or piglet weaned to milk replacer (younger than 3 weeks of age) as a model for infant to study the pathophysiology of *E. coli* F18-diarrhea and to investigate the effect of food/milk components that may protect infants from diarrhea. Results from *ex vivo* study revealed no

Table 2. Haematological Parameters of the Piglets throughout the Experiment

	2 weeks old			3 weeks old			SE	P value		
	d 1	d 8	d 11	d 1	d 8	d 11		Age	Day	Age*day
WBC, log cell number/ L	9.80 <sup>a</sup>	9.98 <sup>b</sup>	10.2 <sup>c</sup>	10.2 <sup>bc</sup>	10.3 <sup>d</sup>	10.3 <sup>d</sup>	1.17	<0.01	<0.01	0.01
Neutrophils <sup>I</sup> , %	43.9 <sup>a</sup>	33.4 <sup>b</sup>	46.1 <sup>b</sup>	62.9 <sup>a</sup>	43.1 <sup>b</sup>	44.5 <sup>b</sup>	7.12	0.11	0.01	0.10
Lymphocytes <sup>I</sup> , %	47.1 <sup>a</sup>	61.2 <sup>b</sup>	47.0 <sup>b</sup>	28.5 <sup>a</sup>	47.2 <sup>b</sup>	47.8 <sup>b</sup>	6.86	0.11	0.01	0.13
Monocytes <sup>I</sup> , %	16.4	2.94	5.92	8.38	9.66	7.67	4.85	0.28	0.44	0.29
Eosinophils <sup>I</sup> , %	0.31	0.32	0.57	0.18	0.47	0.32	0.27	0.05	0.10	0.33
RBC, log cell number/ L	12.7 <sup>a</sup>	12.8 <sup>b</sup>	12.8 <sup>b</sup>	12.7 <sup>a</sup>	12.8 <sup>b</sup>	12.8 <sup>b</sup>	0.03	0.54	<0.01	0.17
Hb, mmol/L	10.6 <sup>a</sup>	12.5 <sup>b</sup>	12.4 <sup>a</sup>	11.2 <sup>a</sup>	12.2 <sup>b</sup>	11.2 <sup>a</sup>	0.64	0.93	<0.01	0.10
Hct, mmol/L	31.8 <sup>a</sup>	37.3 <sup>b</sup>	37.5 <sup>ab</sup>	33.4 <sup>a</sup>	37.2 <sup>b</sup>	35.1 <sup>ab</sup>	2.53	0.75	<0.01	0.48

<sup>I</sup>The values are presented as a percentage of total WBC. WBC: white blood cells. RBC: red blood cells. Hb: hemoglobin. Hct: hematocrit. d: day

difference in term of association of *E. coli* F18 to the intestinal tissue of the 2 and 4 weeks old pigs. Given that association of *E. coli* F18 to the intestinal tissue (mucosa) is facilitated by the specific receptor (F18-receptor) on the mucosal surface, this present result suggests that piglets at 2 weeks of age have had functional/active receptor, mediating the binding of *E. coli* F18 to intestinal mucosa, similar to that of 4 weeks old pigs. At the molecular level, Jensen *et al.* (2012) revealed that piglets younger than 3 weeks of age (indeed neonatal piglets) exhibit active expression of FUT1 gene in their small intestine with no apparent difference when compared with that in the intestine of 4 weeks old pigs. Taken together, it was inferred that suckling piglets possess active F18-receptor on the small intestinal epithelium and, thus are susceptible to *E. coli* F18-diarrhea.

To support the data arising from the *ex vivo* study, it was conducted *in vivo* study involving the piglets weaned from their dams at 2 and 3 weeks of age. In the previous work (Sugiharto *et al.*, 2014), it was noticed that piglets at 4 weeks of age weaned to milk replacer suffered from diarrhea after infection with *E. coli* F18. Based on this and also the results from *ex vivo* study, it was therefore thought not necessary to include 4 weeks old pigs in this current *in vivo* study as the focus was mainly to elucidate the susceptibility of piglets at the age of 3 weeks or younger to *E. coli* F18. The *in vivo* study did not show any

significant differences between the 2 and 3 weeks old pigs with regard to most of the measured responses obtained according to the challenge with *E. coli* F18. It should however be noted that 9 out of 24 piglets refused to drink the milk, and the reason for this could not be identified. Both age groups (weaning at 2 or 3 weeks old) developed diarrhea, indicated by elevated proportion of piglets with diarrhea and decreased DM of the faeces, following inoculation with *E. coli* F18. In order to have enough replications (pens) in the present *in vivo* study no a control group (piglets not inoculated with *E. coli* F18). However, there was confidence that the development of diarrhea in the piglets was attributed to the *E. coli* F18 as all the piglets used in this study were assessed genetically susceptible to *E. coli* F18. Furthermore, the faecal DM and counts of haemolytic *E. coli* remained stable at high and low levels, respectively, in piglets that refused the challenge-inoculum and milk replacer (data not shown), which also supports the sureness. The results in study were in accordance with expectations based on the previous study (Jensen *et al.*, 2012), and may therefore diminish the doubt concerning the absence of *E. coli* F18-diarrhea susceptibility in suckling piglets and piglets younger than 3 weeks of age that has previously been reported (Coddens *et al.*, 2007). In agreement with Jensen *et al.* (2012), *E. coli* F18-diarrhea in pigs may not merely be age

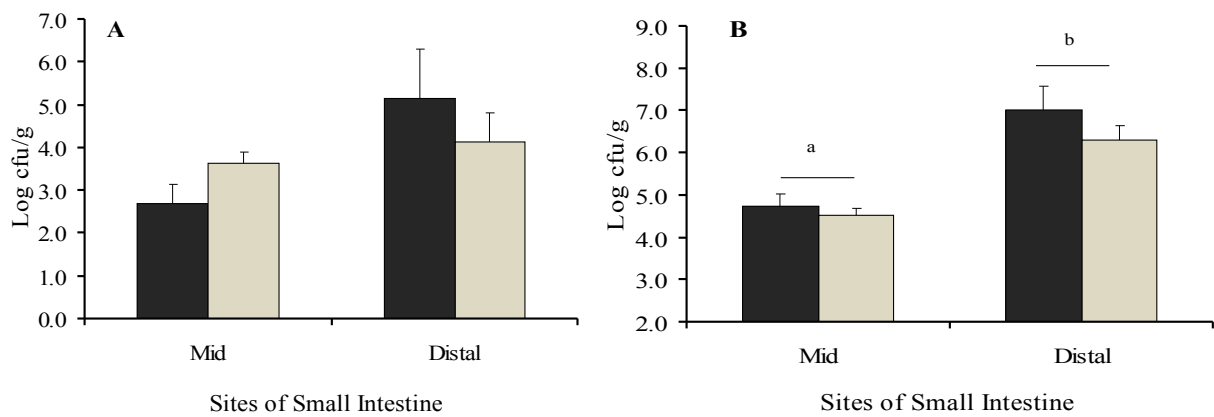


Figure 2. The counts of haemolytic *E. coli* (A) and total coliform (B) associated with the small intestinal tissue of piglets. The different letters in B indicate differences ( $P<0.05$ ) between the sites of the small intestine

dependent, but may also be due to the effect of weaning which implies the loss of passive immunity (maternal antibodies) provided through sow's milk, and thereby impairs the immune reactivity of the piglets against *E. coli* F18. However, it should be noted that the maternal antibodies are at the lowest level on 10–18 days after farrowing, while the piglets do not normally reach the mature immune system at 2–3 weeks of age (Thacker, 1999). Taken together, the less own immunocompetence of the piglets at 2 or 3 weeks of age in the current study may contribute to a greater extent to the development of *E. coli* F18-diarrhea, as compared to the effect of the loss of maternal antibody due to weaning.

In the present study, *E. coli* F18 inoculation was noticed to raise the numbers of WBC and RBC, lymphocyte fractions and the concentrations of Hb and Hct in the blood of piglets. The corresponding results were reported by Peltola *et al.* (2006) and Yates *et al.* (2011) who found increased WBC in the humans after *E. coli* infection and increased lymphocyte fractions, RBC, Hb and Hct in ewes after lipopolysaccharide injection, respectively. Aside from the effect of bacterial infection, the counts of WBC may depend on the age of piglets, in which older pigs have higher counts of WBC than younger ones (Davis *et al.*, 2006). Hence, it was difficult to explain the observed difference in counts of WBC and faecal population of haemolytic *E. coli* between the age groups in this study. Neutrophil is a type of WBC that is

important in the host defense against bacterial infections. Concomitant with WBC, the concentration of neutrophils has been reported to be higher in older pigs than in younger pigs (David *et al.*, 2006; Sugiharto *et al.*, 2014). Beyond the age-related difference, the present investigation showed that the proportion of neutrophils to the total WBC decreased with *E. coli* F18 inoculation. The exact rationale for this condition was not clearly elucidated, but neutrophil migration from the circulation across the endothelium into the intestine (Stadnyk *et al.*, 2000) in response to *E. coli* F18 infection may be responsible for the decreased concentration of neutrophils in the blood.

With regard to the common ETEC inoculation procedure, it has generally been considered that insertion of an orogastric tube is laborious and may cause discomfort and pain for the small piglets (Pandey *et al.*, 2013). Hence, inoculation of *E. coli* through the milk replacer could be a more animal- and human friendly way of performing such experiments. Based on the results, inoculation through provision of milk replacer could be applied as an alternative to induce diarrhea when conducting the infection studies with the suckling piglets. Yet, it should be conducted with caution as the aversion of the piglets to consume milk replacer (containing challenge inoculum) may limit the success in the experimental induction of *E. coli*-diarrhea. Further studies are needed to deal with this problem.

## CONCLUSIONS

The age has no influence on the susceptibility of piglets to *E. coli* F18-diarrhea. Piglets younger than 3 weeks of age (fed milk replacer) could be employed to model the *E. coli* F18-diarrhea progression in infant.

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